09/023483

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Term: L4 and random primer$1

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Search History

DATE: Thursday, May 15, 2003 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L5	L4 and random primer$1	1	L5
L4	determin$3 total DNA or measur$3 total DNA or detect$3 total DNA	33	L4
L3	L2 and (nucleotide near5 bind$3)	1	L3
L2	L1 and random primer$1	13	L2
DB=DWPI,USPT,EPAB,JPAB; PLUR=YES; OP=ADJ			
L1	(measur$3 or determin$3) near5 total near5 DNA	261	L1

END OF SEARCH HISTORY

End of Result Set

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L5: Entry 1 of 1 File: USPT Aug 11, 1992

DOCUMENT-IDENTIFIER: US 5137816 A
TITLE: Rhizobial diagnostic probes and rhizobium trifolii nifH promoters

Detailed Description Text (81):
In strain ANU843, the initially-isolated copy of the RDS, RS-1(b), was localized to a 1 kb region close to the 5' end of nifH. At least five copies of the RDS were found exclusively on the Sym plasmid of ANU843. No such sequence was detected in total DNA isolated from ANU845, a Sym plasmid-cured derivative of ANU843. A second copy of the RDS, RS-3, has been mapped approximately 28 kb distant from nifH. The uniqueness of this repeated sequence was shown by the fact that there was no detectable hybridization of this repeated sequence to the DNA of other rhizobial species, e.g., R. meliloti or R. leguminosarum. These two copies of the RDS, which have been mapped, flank a region which carries all of the identified nodulation and nitrogen fixation genes. This observation together with the fact that all copies of the RDS of R. trifolii are located on the Sym plasmid suggest that such sequences may play a role in the specificity of host-symbiont interaction during nodulation. Since an RDS is unique to rhizobial isolates that infect and nodulate a specific legume family, it can be used to quickly and efficiently identify other similar bacterial strains regardless of their origin. In principle, a recombinant plasmid comprising RDS (RDS plasmid, hereinafter) is constructed and amplified by replication in a host bacterial cell. Any host cell strain in which the RDS plasmid replicates is suitable for maintaining the plasmid and for generating adequate quantities of plasmid DNA. The plasmid itself may be derived from any stably-replicating plasmid vector capable of multicopy replication and bearing a genetic marker, such as a drug resistance gene, to permit selection of host cell lines carrying the plasmid.

Detailed Description Text (106):
Hybridization probes were prepared by the following and similar procedures all as known to the art, using primed synthesis with DNA polymerase I (Klenow fragment) using denatured random calf thymus DNA primers. Linearized plasmid DNA (100 mg) was heat-denatured by boiling for 2 minutes with 100 .mu.g random primers (8-12 nucleotide fraction of DNAse I-treated calf thymus DNA) in 20.mu.l and cooled on ice for 30 seconds. Denatured DNA was incubated for 30 minutes at 37.degree. C. with 1 unit DNA polymerase I (Klenow fragment) in 10 mM Tris-HCl pH 7.4, 7-8 mM MgCl.sub.2, 10 mM .beta.-mercaptoethanol, 600 .mu.M each of dGTP, dATP, and dTTP and 0.3 .mu.M .alpha.-.sup.32 P-dCTP (>3000 Ci/mmol, Amersham). The reaction was stopped by phenol/chloroform extraction and the aqueous phase passed over a Sephadex G-50 column to remove unincorporated radioactivity. Peak fractions (specific activity 107-108 cpm/.mu.g DNA) were precipitated by the addition of E. coli tRNA (20 .mu.g) and 2.5 vol. ethanol at -20.degree. C.